Applicant: Richard M. Broglie et al. Attorney's Docket No.: 07148-025003

/ CGL99/0008US04

Serial No.: 09/643,579 Filed: August 22, 2000

Page : 2 of 17

## Amendments to the Specification:

Please replace the Sequence Listing filed on August 22, 2000, with the following substitute Sequence Listing.

## In the Title:

Please replace the title with the following rewritten title:

METHODS FOR INCREASING OLEIC ACID CONTENT IN SEEDS FROM TRANSGENIC PLANTS CONTAINING A MUTANT DELTA 12 DESATURASE

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Serial No.: 09/643,579 Filed : August 22, 2000

Page : 3 of 17

Please replace Table 1 at page 17, with the following amended Table 1:

## TABLE 1 Amino Acid Sequence Conserved Between Plant Microsomal Delta-12 Desaturases and Microsomal and Plastid Delta-15 Desaturases

	Conserved AA Positions in	Consensus	Consensus	
	SEQ ID NO:2	Conserved AA	Conserved AA	
	of USSN	Sequence in	Sequence in	Consensus
Region	08/262,401	$\Delta^{12}$ Desaturases	$\Delta^{15}$ Desaturases	AA Sequence
A	39-44	AIPPHC	AIPKHC	AIP(P/K)HC
		(SEQ ID NO:18)	(SEQ ID NO:19)	(SEQ ID NO:20)
В	86-90	WP(L/I)YW	WPLYW	WP(L/I)YW
		(SEQ ID NO:21)	(SEQ ID NO:22)	(SEQ ID NO:21)
C	104-109	AHECGH	GHDCGH	(A/G)H(D/E)CGH
		(SEQ ID NO:23)	(SEQ ID NO:24)	(SEQ ID NO:25)
D	130-134	LLVPY	ILVPY	(L/I)LVPY
		(SEQ ID NO:26)	(SEQ ID NO:27)	(SEQ ID NO:28)
E	137-142	WKYSHR	WRISHR	W(K/R)(Y/I)SHR
		(SEQ ID NO:29)	(SEQ ID NO:30)	(SEQ ID NO:31)
F	140-145	SHRRHH	SHRTHH	SHR(R/T)HH
		(SEQ ID NO:32)	(SEQ ID NO:33)	(SEQ ID NO:34)
G	269-274	ITYLQ	VTYLH	(I/V)TYL(Q/H)
		(SEQ ID NO:35)	(SEQ ID NO:36)	(SEQ ID NO:37)
Н	279-282	LPHY	LPWY	LP(H/W)Y
		(SEQ ID NO:38)	(SEQ ID NO:39)	(SEQ ID NO:40)
I	289-294	WL(R/K)GAL	YLRGGL	(W/Y)L(R/K)G(A/G)L
		(SEQ ID NO:41)	(SEQ ID NO:42)	(SEQ ID NO:43)
J	296-302	TVDRDYG	TLDRDYG	T(V/L)DRDYG
		(SEQ ID NO:44)	(SEQ ID NO:45)	(SEQ ID NO:46)
K	314-321	THVAHHLF	THVIHHLF	THV(A/I)HHLF
		(SEQ ID NO:47)	(SEQ ID NO:48)	(SEQ ID NO:49)
L	318-327	HHLFSTMPHY	HHLFPQIPHY	HHLF(S/P)(T/Q)(I/M)PHY
		(SEQ ID NO:50)	(SEQ ID NO:51)	(SEQ ID NO:52)

Applicant: Richard M. Broglie et al.

Serial No.: 09/643,579

Attorney's Docket No.: 07148-025003

/ CGL99/0008US04

Serial No.: 09/643,579 Filed: August 22, 2000

Page : 4 of 17

Please replace Table 2 at page 29, with the following amended Table 2:

TABLE 2
Alignment of Amino Acid Sequences of Cloned Canola
Membrane Bound-Desaturases

Desaturase Gene	Sequence <sup>a</sup>		Position <sup>b</sup>
Canola-FAD2-D	HECGH (SEQ ID NO:53)	110	
Canola-FAD2-F	HECGH (SEQ ID NO:53)	110	
Canola-FAD6 <sup>c</sup>	HDCAH (SEQ ID NO:54)	171	
Canola-FAD3 <sup>d</sup>	HDCGH (SEQ ID NO:55)	97	
Canola-FAD7 <sup>e</sup>	HDCGH (SEQ ID NO:55)	126	

<sup>&</sup>lt;sup>a</sup>One letter amino acid code; conservative substitutions are underlined

Please replace the paragraph beginning at page 30, line 27 through page 31, line 23, with the following amended paragraph:

## EXAMPLE 3 CONSTRUCTS FOR DOMINANT NEGATIVE SUPPRESSION OF DELTA-12 FATTY ACID DESATURASE

The vector pZS212 was used to construct plasmids for dominant negative suppression experiments. One construct was prepared by inserting the full-length mutant D gene coding sequence (nucleotides 1 to 1155 of SEQ ID NO:3) in sense orientation between the phaseolin promoter and phaseolin 3' poly A region of plasmid pCW108. The pCW108 vector contains the bean phaseolin promoter and 3' untranslated region and was derived from the commercially available pUC18 plasmid (Gibco-BRL) via plasmids AS3 and pCW104. Plasmid AS3 contains 495 base pairs of the bean (Phaseolus vulgaris) phaseolin (7S seed storage protein) promoter starting with 5'-TGGTCTTTTGGT-3' (SEQ ID NO:56) followed by the entire 1175 base pairs of the 3' untranslated region of the same gene (see sequence descriptions in Doyle et al., (1986) *J. Biol. Chem.* 261:9228-9238 and Slightom et al., (1983) *Proc. Natl. Acad. Sci. USA*, 80:1897-1901. Further sequence description may be found in WO 9113993) cloned into the Hind III site

<sup>&</sup>lt;sup>b</sup>Position in gene product of first amino acid

<sup>&</sup>lt;sup>c</sup>FAD6 = Plastid delta-12

<sup>&</sup>lt;sup>d</sup>FAD3 = Microsomal delta-15

<sup>&</sup>lt;sup>e</sup>FAD7 = Plastid delta-15

Applicant: Richard M. Broglie et al. Attorney's Docket No.: 07148-025003

/ CGL99/0008US04

Serial No.: 09/643,579 Filed: August 22, 2000

Page : 5 of 17

of pUC18. The additional cloning sites of the pUC18 multiple cloning region (Eco RI, Sph I, Pst I and Sal I) were removed by digesting with Eco RI and Sal I, filling in the ends with Klenow and religating to yield the plasmid pCW104. A new multiple cloning site was created between the 495bp of the 5' phaseolin and the 1175bp of the 3' phaseolin by inserting a dimer of complementary synthetic oligonucleotides consisting of the coding sequence for a Nco I site (5'-CCATGG-3') followed by three filler bases (5'-TAG-3'), the coding sequence for a Sma I site (5'-CCGGG-3'), the last three bases of a Kpn I site (5'-TAC-3'), a cytosine and the coding sequence for an Xba I site (5'-TCTAGA-3') to create the plasmid pCW108. This plasmid contains unique Nco I, Sma I, Kpn I and Xba I sites directly behind the phaseolin promoter.

Please replace the paragraph beginning at page 33, line 10, with the following amended paragraph:

• Sac I site (5'-GAGCTC-3') followed by more additional bases (5'-GTCGACGAGG-3') (SEQ ID NO:57). The 5' end of BR58 had additional bases (5'-GAGCTC-3') followed by bases corresponding to a Nco I site (5'-CCATGG-3') followed by additional bases (5'-AGATCTGGTACC-3') (SEQ ID NO:58).